Adipokines and myokines are important for several biological effects of adipose tissue and skeletal muscle as well as health

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Public health
- Diet
- Physical activity
- Non-smoking
Myokines & adipokines ➔
health & disease

The adipose organ

Cinti S: The adipose organ, 1999
Editrice Kurtis, Milano Italy

C57BL mouse; 44 % of the adipose organ is anterior (dorsal subcutaneous interscapular +) & ventral (dorsolumbar, inguinal & gluteal)

Most WAT

BAT in interscapular, inguinal, mediastinal & perirenal regions
The adipose organ at a glance
Saverio Cinti

**Adipose organ composition is dynamic**

Evidence suggests that adipocyte transdifferentiation might underlie the changes in adipose organ composition that are observed in response to chronic cold or exercise, caloric excess or pregnancy/ lactation. In vivo, adipocyte development is also associated with these conditions. The factors driving these transdifferentiation pathways are under investigation, but might include those detailed in Box 1 (see main text).

**Cytology: the adipose organ contains many cell types**

- **Adipocytes**
  - Brown
  - UCP1<sup>+</sup> leptin<sup>−</sup> S100-B
  - Pseudocellular<sup>+</sup>
  - UCP1<sup>−</sup> leptin<sup>+</sup> S100<sup>−</sup> B
  - Small white
  - UCP1<sup>−</sup> leptin<sup>−</sup> S100<sup>−</sup> B
  - Large white
  - UCP1<sup>−</sup> leptin<sup>−</sup> S100<sup>−</sup> B
  - Adipocyte-derived endothelial cell
  - UCP1<sup>−</sup> leptin<sup>−</sup> WAP
  - Immune cells
  - Lymphocyte
  - Various (those in C57 are MAC2)

**Adipose organ pathology contributes to insulin resistance**

Obesity causes expansion of the adipose organ by increasing the number and size of white adipocytes. On reaching a critical size, white adipocytes are prone to cell death. The obese adipose organ, and particularly visceral fat, is infiltrated with macrophages, which form crown-like structures surrounding dead and dying adipocytes. These macrophages produce pro-inflammatory cytokines that trigger the systemic circulation and contribute to the development of insulin resistance. Microscopic image from Cinti et al., 2000, with permission.

Abbreviations: BAT, brown adipose tissue; IL-3, interleukin-3; IL-6, interleukin-6; MAC2, also known as LGALS3 (galactin-3); S100-B, S-100 protein beta chain; SNS, sympathetic nervous system; TNFα, tumor necrosis factor-alpha; UCP1, uncoupling protein 1; WAP, whey acidic protein; WAT, white adipose tissue.
Adipose tissue size & location

• Very large 3 - 70 % of body mass
  – May store energy for several months fasting

• Location is important
  – Subcutaneous; protect, insulate
  – Omental; ectopic, insulin resistance
  – Perirenal; protect
  – Epididymal; essential fatty acids
  – Interscapular; BAT, heat
  – Inguinal, axillar; essential fatty acids
  – Retrobulbar; support
Adipose tissue (AT) depots

- ~ 85% of total AT is subcutaneous, in lean or obese humans
- The remaining 15% is intra-abdominal, including visceral & retroperitoneal depots
- Visceral AT (mesenteric and omental), only constitutes ~ 10% of total body fat, with the highest risk for metabolic dysregulation
Figure 1. Description of body fat distribution in humans. Lower body: fat storage around the buttocks, hips and thighs. Abdominal subcutaneous: subcutaneous fat storage around the stomach and chest. Overall coverage: fat accumulation in the arms, breast, thighs, buttocks, lower back and breast. Visceral: intra-abdominal fat deposition among organs such as the intestines, stomach, liver and pancreas. Fat distributed within the visceral cavity is highly associated with obesity-related health consequences whereas other fat distribution is not.
Metabolic alterations following visceral fat removal and expansion

Foster & Pagliassotti. *Adipocyte* 2012, 1, 192-9

**Figure 2.** Differences between visceral and subcutaneous adipose tissue depots. Drain location: the visceral depot (left) releases products into the portal vein, while the subcutaneous depot (right) releases products into the systemic circulation. In obesity, portal vein effluent to the liver contains higher concentrations of free fatty acids and interleukin-6 compared with the systemic circulation. Adipose depot: Visceral and subcutaneous fat are characterized by inherent differences. When compared with subcutaneous fat, visceral fat is characterized by reduced adiponectin and leptin, increased inflammatory adipocyte/cytokines, enhanced lipolysis, a reduced response to insulin and reduced differentiation and angiogenesis.
Adipose tissue functions

- **Storage of**
  - energy - very large & efficient
  - cholesterol, vitamin D & E
- **“Insulation”** - thermic, mechanical & (electrical)
- **Regulation of metabolism** - white & brown
- **Adipokines** – auto-/para-/endocrine
Energy excess

A. Functional triglyceride storage

- Glucose
- Lipokines
- Adipokines (adaptive)

Excess lipids (Gut, liver)

Fatty acids (Fuel on demand)

Fatty acids → Ceramides

De novo lipogenesis

ACC

MGL

HSL

Insulin

ATGL

Adipocyte

Triglycerides

Lipid droplet

B. Impaired triglyceride storage

- Glucose
- Lipokines
- Adipokines (adverse)

Excess lipids (Increased flux)

Fatty acids

Ceramides

De novo lipogenesis

ACC

MGL

HSL

Insulin resistance

ATGL

Adipocyte

Triglycerides

Lipid droplet

Systemic effects
- Ectopic lipids
- Lipotoxicity
- Inflammation
- Insulin resistance
- Gluconeogenesis
FGF21 and Slit2-C may target WAT browning WAT. FGF21, IL-6 (and ANGPTL8) may improve insulin secretion and β-cell function. NRG4 attenuates hepatic lipogenesis; insulin-like growth factor binding protein 2 (IGFBP2) promotes bone formation; FGF21 and IL-6 may increase cardiac substrate oxidation. BATokines might modulate systemic metabolism indirectly through the CNS; FGF21, IL-6 (and BMP8b) may influence sympathetic activity, feeding, circadian behaviour and female endocrine function. IGF1, insulin-like growth factor 1; NRG4, neuregulin 4.
Leptin expression

- Adipose tissue - white & brown
- **Placenta** (Hassink et al. Pediatrics. 1997; 100 (1):E1)
- **Fetus - hair follicles, bone/cartilage** (Hoggard et al. PNAS. 1997; 94:11073-11078)
- **Gastric epithelium** (Bado et al. Nature. 1998, 394, 790-793)
- **Breast gland epithelium** (Casabiell et al. J Clin Endocrinol Metab. 1997, 82, 4270-4273)
- **Skeletal muscle** (Wang et al. Nature. 1998, 393, 684-648)
Expression of leptin & leptin receptor (OB-R) mRNA in human osteoblasts

RT-PCR products

Leptin

OB-\(R_b\)

OB-\(R_{ex}\)

G3PDH

BeWo  hOB  NHOb  788T  KPDXM  OHS  WAT  hOB

Osteoblasts  Osteosarcomas

Endocrine effects of leptin on bone metabolism

↑ Proliferation
↑ Bone mineralization
↑ Collagen deposition
≈ Leptin
≈ Leptin receptor

Bone tissue adapts to the amount of adipose tissue
Effect of energy restriction (diet) & physical exercise on mRNA from adipose tissue and whole body MRI
Lee et al. *Phys Report* 2016, 4 (21) e13019

- **Exercise** group used 15,800 kcal/w, increasing to 18,400 kcal during intervention (~17% increase; MyoGlu)

- **Diet** group used 14,500 kcal/w; loss in body weight (75% AT & 25% fat free mass \(\rightarrow\) 2600 kcal/w (18% reduction; NutriTech)

- Thus, similar energy alterations
Table 3. Changes in fat depots after 12 weeks exercise and energy restriction (diet).\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Exercise Change (%)</th>
<th>Diet Change (%)</th>
<th>Exercise, control Change (%)</th>
<th>Control Change (%)</th>
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<tbody>
<tr>
<td>MRI</td>
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<tr>
<td>Total AT</td>
<td>–10.9 ± 5.1*</td>
<td>–8.9 ± 3.3*</td>
<td>–8.5 ± 2.7*</td>
<td>3.7 ± 1.4</td>
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<tr>
<td>Subcutaneous AT</td>
<td>–7.3 ± 6.0*</td>
<td>–8.6 ± 3.7*</td>
<td>–6.6 ± 2.6*</td>
<td>2.0 ± 1.0</td>
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<tr>
<td>Intra-abdominal AT</td>
<td>–19.4 ± 10.8*</td>
<td>–11.4 ± 6.2*</td>
<td>–16.9 ± 4.2*</td>
<td>7.7 ± 3.3</td>
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<tr>
<td>MRS fat</td>
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<tr>
<td>Pancreas(^3)</td>
<td>–28.5 ± 62.9</td>
<td>–20.8 ± 49.7</td>
<td>–30.3 ± 21.7</td>
<td>21.3 ± 20.6</td>
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<tr>
<td>Liver(^4)</td>
<td>–27.4 ± 15.7*</td>
<td>–7.4 ± 2.4*</td>
<td>–23.3 ± 14.1(^5)</td>
<td>–6.8 ± 1.9*</td>
</tr>
</tbody>
</table>

\(^1\)Data represent mean ± SEM. Only relative values are presented due to slight differences in protocols and units calculated in the two cohorts.

\(^2\)Only data from six subjects in the diet group were available.

\(^3\)\(n = 7\) in the exercise group.

\(^4\)\(n = 9\) in the exercise group.

\(^5\)The reduction in the control group is significant using the Wilcoxon test (Langleite et al. 2016).

\(*P < 0.05\) (baseline vs. 12 weeks).

Markedly more AT loss with exercise
<table>
<thead>
<tr>
<th>Pathway</th>
<th>Exercise</th>
<th>Diet</th>
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<tr>
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<td>P-value</td>
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<tr>
<td>Immune-related pathways</td>
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<td>Chemokine signaling pathway</td>
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<td>Osteoclast differentiation</td>
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<td>Complement and coagulation cascades</td>
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<td>NOD-like receptor signaling pathway</td>
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<td>Jak-STAT signaling pathway</td>
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<td>Natural killer cell mediated cytotoxicity</td>
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<td>T-cell receptor signaling pathway</td>
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<td>B-cell receptor signaling pathway</td>
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<td>Leukocyte transendothelial migration</td>
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<td>Energy-related pathways</td>
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<td>Glycolysis/gluconeogenesis</td>
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<td>Fatty acid metabolism</td>
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<td>Alanine, aspartate and glutamate metabolism</td>
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<td>Pyruvate metabolism</td>
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<td>Peroxisome</td>
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<td>Insulin signaling pathway</td>
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</table>
A) **Reduced** mRNA expression of 12 of 18 markers of adipose tissue M2-like macrophages after exercise

B) **Increased** mRNA expression of 7 of 18 markers of adipose tissue M1-like macrophages after energy restriction
Energy restriction vs exercise

Lee et al. Phys Report 2016, 4 (21) e13019

- **Energy restriction**
  - **Increased lipolysis**
  - **Increased expression of markers of M1-like macrophages in AT**
    - M1 "killer" macrophages activated by LPS and IFN-g, secrete high amounts of IL-12 & low amounts of IL-10

- **Exercise**
  - **Reduce expression of markers of M2-like macrophages & T cells**
    - M2 → wound healing & tissue repair; turn off immune activation via anti-inflammatory cytokines like IL-10
    - Resident tissue macrophages can be further elevated by IL-4
    - High levels of IL-10, TGF-b & low levels of IL-12
    - Tumor-associated macrophages are mainly M2, promote tumor growth
Exercise and Regulation of Adipokine and Myokine Production

Sven W. Görgens*, Kristin Eckardt†, Jørgen Jensen‡, Christian A. Drevon†, Jürgen Eckel*§"}

Figure 1 The adipo-myokine concept. A search of original articles in PubMed was performed for the major exercise-regulated myokines and adipokines to identify molecules that were produced and secreted in both tissues. The term adipo-myokines was used for proteins fulfilling both of these criteria. The search terms we used were “skeletal muscle” or “adipose tissue,” “myokine” or “adipokine,” and “exercise.”
Physical activity protects against chronic disorders

- **CVD**; Thompson *ATVB* 2003, **23**, 1319-21
- **T2D**; Knowler et al. *NEJM* 2002, **346**, 393-403
- **Dementia**; Lautenschlager et al. *JAMA* 2003, **300**, 1027-37
- **Cancer**; *WCRF report* 2007, colorectal, breast, prostate
Contraction-induced signals like IL-6

IL-6 from AT different skeletal muscle?

Pedersen BK et al. Physiol Rev. 2008
Our strategy for identifying novel myokines

Human grown myotubes in culture; 6 h - FCS

Targeted immunoassay

Proteomic analyses of secretome

Concentration by membrane cut-off 3 kD
Separation by 1-D SDS-PAGE
Collection of 10 “bands”, Trypsine
MALDI-TOF MS
Data sorting

Hit list including novel myokines

Hit list including ~ 20 most abundant proteins

Validity/ Physiological relevance?

Time-course
Expression detected by RT-PCR & Western
Expression during differentiation of satellite cells to myotubes
Regulated expression/ secretion?
Effect of recombinant peptide in vitro? Paracrine?
Expressed in skeletal muscle tissue?
Expression altered by exercise/ inactivity/ wasting in vivo?
Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training

- **236 proteins** detected by proteomics in medium from cultured human myotubes
- **18 classically secreted proteins expressed in skeletal muscle**, using the SignalP 3.0 and Human Genome Expression Profile databases together with a published mRNA-based reconstruction of the human skeletal muscle secretome
- **17 of the secreted proteins** exhibited mRNA expression in cultured human myotubes and skeletal muscles biopsies
- **15 of these** had significantly **enhanced mRNA expression** in *m. vastus lateralis* and/or *m. trapezius* after 11 wk of strength training
<table>
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<tr>
<th>Protein Name</th>
<th>QMa</th>
<th>Scoreb</th>
<th>MWc</th>
<th>Myotubes: mRNAd</th>
<th>VL: mRNAe</th>
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</table>

aQM denotes the number matched queries. bScore denotes the proteins’ MASCOT scores. cTheoretical molecular mass of proteins is displayed according to the UniProtKB/ Swiss-Prot entry (mass; kDa). dMean mRNA expression from three donors is presented as normalized to RPLP0. eMean mRNA expression in m. vastus lateralis biopsies from 10 healthy male subjects normalized to RPLP0.
<table>
<thead>
<tr>
<th>Protein Name</th>
<th>M. vastus lateralis</th>
<th>M. trapezius</th>
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<tr>
<td>Collagen alpha-1(I) chain</td>
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<td>4.7 (2.5–18.5)*</td>
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<td>Lumican</td>
<td>2.5 (1.7–3.7)*</td>
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<td>Fibronectin 1</td>
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<td>Cathepsin H</td>
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<td>1.2 (1.0–2.0)</td>
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</table>
Single exercise bout vs. Chronic exercise training

- Single exercise bout (<3 h):
  - Secretion of muscle-derived adipo-myokines
    - IL-6
    - LIF
    - IL-7
    - Fstl1
    - etc.
  - Muscle repair and growth
  - Endothelial function and angiogenesis
  - Adipocyte lipolysis
  - Hepatic glucose release into circulation
  - Inflammation

- Chronic exercise training (>8 weeks):
  - Reduction of adipose tissue-derived pro-inflammatory adipo-myokines
    - IL-6
    - TNFα
    - MCP-1
    - Leptin
    - etc.
  - Insulin sensitivity
  - Physical fitness
  - Visceral fat mass
  - Inflammation
Plasma concentration of IL-6 (pg/mL)


**CONTROLS AT BASELINE**

**CONTROLS AFTER 12 WEEKS TRAINING**

**PREDIABETICS AT BASELINE**

**PREDIABETICS AFTER 12 WEEKS TRAINING**

Significant increase in IL-6 after ACUTE exercise (both at 0 and 2 h)  \( p < 0.01 \), but NOT after 12 w training intervention
After 45 min cycling ~ 550 genes were upregulated
  – 28 genes (5%) were directly related to ECM
Long-term exercise (12 w) enhanced expression of 289 genes >50%
  – 20% were ECM related
> 50% of the proteoglycans in muscle were significantly enhanced after 12 w
Secretion of the PG serglycin for the first time from SKM
SRGN KO \(\rightarrow\) enhanced expression & secretion of serpin E1 (SERPINE1; serine proteinase inhibitor superfamily. Inhibitor of tissue plasminogen activator (tPA) and urokinase (uPA) \(\rightarrow\) inhibitor of fibrinolysis)
Belgian blue – mutated gene encoding myostatin inhibiting muscle growth
Myostatin & exercise in humans

- Myostatin mRNA expression reduced in SKM after acute & long-term PA
- Even further reduced by acute exercise on top of 12 w training
- Expression of myostatin at baseline correlated negatively with insulin sensitivity
- Myostatin expression in AT increased after 12 w training
  - correlated positively with insulin sensitivity markers
- In cultured SKM cells but not in SGBS cells, myostatin promoted insulin-independent increase of glucose uptake
- SKM cells incubated with myostatin enhanced glucose oxidation & lactate production
- Myostatin differentially expressed in muscle (-) and AT (+) in relation to PA and dysglycaemia. Recombinant myostatin increased consumption of glucose in human skeletal muscle cells, suggesting a role of myostatin in skeletal muscle glucose metabolism
Table 2: SignalP-positive genes encoding secretory proteins that were downregulated more than 1.5 times (FC < 0.667) in the skeletal muscle during 12 weeks of training (n = 26)

<table>
<thead>
<tr>
<th>Genes</th>
<th>Gene symbol</th>
<th>FPKM*</th>
<th>Fold change†</th>
<th>P-value‡</th>
<th>q-value§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuronal pentraxin 1</td>
<td>NPTX1</td>
<td>0.2</td>
<td>0.26</td>
<td>2E−08</td>
<td>3E−07</td>
</tr>
<tr>
<td>Cadherin 22, type 2</td>
<td>CDH22</td>
<td>0.2</td>
<td>0.39</td>
<td>3E−07</td>
<td>4E−06</td>
</tr>
<tr>
<td>Gremlin 2, DAN family BMP antagonist</td>
<td>GREM2</td>
<td>2.6</td>
<td>0.46</td>
<td>2E−05</td>
<td>1E−04</td>
</tr>
<tr>
<td>Protease, serine, 50</td>
<td>PRSS50</td>
<td>1.7</td>
<td>0.53</td>
<td>9E−07</td>
<td>9E−06</td>
</tr>
<tr>
<td>Olfactomedin 1</td>
<td>OLFM1</td>
<td>4.6</td>
<td>0.58</td>
<td>1E−11</td>
<td>4E−10</td>
</tr>
<tr>
<td>Myostatin</td>
<td>MSTN</td>
<td>3.8</td>
<td>0.58</td>
<td>3E−06</td>
<td>2E−05</td>
</tr>
<tr>
<td>Toll-like receptor 9</td>
<td>TLR9</td>
<td>0.6</td>
<td>0.59</td>
<td>4E−13</td>
<td>2E−11</td>
</tr>
<tr>
<td>Leucine-rich repeat containing 3B</td>
<td>LRRC3B</td>
<td>4.2</td>
<td>0.62</td>
<td>4E−03</td>
<td>1E−02</td>
</tr>
<tr>
<td>CD274 molecule</td>
<td>CD274</td>
<td>2.2</td>
<td>0.63</td>
<td>3E−13</td>
<td>1E−11</td>
</tr>
<tr>
<td>Shisa family member 3</td>
<td>SHISA3</td>
<td>0.6</td>
<td>0.63</td>
<td>3E−02</td>
<td>6E−02</td>
</tr>
<tr>
<td>LAMB3</td>
<td>LAMB3</td>
<td>2.6</td>
<td>0.64</td>
<td>5E−06</td>
<td>4E−05</td>
</tr>
<tr>
<td>Carboxylesterase 1</td>
<td>CES1</td>
<td>0.9</td>
<td>0.65</td>
<td>1E−05</td>
<td>8E−05</td>
</tr>
<tr>
<td>Na+/K+-transporting ATPase interacting 1</td>
<td>NKAIN1</td>
<td>7.6</td>
<td>0.65</td>
<td>5E−16</td>
<td>4E−14</td>
</tr>
<tr>
<td>Rho GDP dissociation inhibitor (GDI) gamma</td>
<td>ARHGDI</td>
<td>0.7</td>
<td>0.66</td>
<td>2E−02</td>
<td>6E−02</td>
</tr>
<tr>
<td>Chondroadherin</td>
<td>CHAD</td>
<td>6.1</td>
<td>0.66</td>
<td>8E−07</td>
<td>9E−06</td>
</tr>
</tbody>
</table>

*Gene expression level at baseline, measured by mRNA sequencing and expressed as fragments per kilobase of transcript per million mapped reads (FPKM).
†mRNA expression after 12 weeks of training as compared to baseline of the intervention.
‡P-value generated in EdgeR.
§False discovery rate.
Another strategy is to use whole body intervention to discover new myokines
- Tests before and after training (top)
- Acute 45 min bicycle test at 70% of VO\textsubscript{2}max (bottom)
- Blood & muscle samples taken before (B), just after (0’), and 2 h after the acute bout (2 h)
- Subcutaneous adipose tissue biopsies taken 30–60 min after acute exercise

26 middle-aged, sedentary
→ endurance and strength training for 12 w
Global mRNA sequencing of human skeletal muscle: Search for novel exercise-regulated myokines
Pourteymour et al. *Mol Metab.* 2017, 6, 352-65

- **161** secretory transcripts enhanced (>1.5-fold) after acute exercise & **99** increased after 12 w
- **92** secretory transcripts were reduced after acute and/or long-term physical activity
- Selected **17 unknown** myokines sensitive to short- and/or long-term exercise
- Expression also in cultured human skeletal muscle cells
- One of the 17 candidates was macrophage colony-stimulating factor-1 (CSF1)
- CSF1 mRNA increased in skeletal muscle after acute and long-term exercise, accompanied by a rise in plasma CSF1 protein
- In cultured muscle cells, electrical pulse stimulation (EPS) increased expression and secretion of CSF1
- **Conclusion:** 17 new exercise-responsive myokines. **CSF1** responded to EPS in cultured muscle cells; up-regulated in muscle and plasma after acute & long-term exercise. **Marker of exercise?**
Venn diagrams showing the number of secretory genes that were up- or down-regulated >1.5-fold

Pourteymour et al. Mol Metab. 2017, 6, 352-65

Genes up-regulated >1.5 fold

A. Immediately after acute exercise
   - A2/A1: 9
   - B2/B1: 20
   - Total: 117 genes

B. 2 h after acute exercise
   - A3/A1: 42
   - B3/B1: 8
   - Total: 91 genes

C. Immediately or 2 h after acute exercise
   - A2/A1: 70
   - B3/B1: 44
   - Total: 161 genes

D. Acute or 12 w exercise
   - A2/A1: 149
   - B1/A1: 87
   - Total: 248 genes

Genes down-regulated >1.5 fold

E. Immediately after acute exercise
   - A2/A1: 7
   - B2/B1: 10
   - Total: 24 genes

F. 2 h after acute exercise
   - A3/A1: 35
   - B3/B1: 10
   - Total: 70 genes

G. Immediately or 2 h after acute exercise
   - A2/A1: 17
   - B3/B1: 63
   - Total: 87 genes

H. Acute or 12 w exercise
   - A2/A1: 83
   - B1/A1: 5
   - Total: 92 genes
mRNA expression of selected genes in skeletal muscle biopsies (in A1) or cultured human myotubes. mRNA expression in biopsies was determined with RNAseq (n=26), and in myotubes by RT-PCR (n=5-6).
A) mRNA expression of CSF1 and B) CSF1 receptor (CSF1R) in skeletal muscle biopsies at baseline (A1/A3) and after 12 w (B1eB3), *p < 0.05 vs. A1, $ p < 0.05 vs. B1. C) Plasma CSF1 before and after 12 w intervention. D) Differentiating human skeletal muscle cells. E) CSF1 conc in culture medium. Pourteymour et al. *Mol Metab.* 2017, 6, 352-65
Major findings after 12 weeks training
Langleite et al. *Arch Physiol Biochem.* 2016, 122, 167-79

- Increased VO$_2$max $\sim$15 %
- Increased GIR $\sim$30 %
- Dysglycemics reduced body weight (-1.7 kg; $p<0.05$) and waist circumference (-3.7 cm; $p<0.01$)
- Visceral fat preferentially lost compared to other ATdepots
- Hepatic fat was 5-fold higher in dysglycemics than controls, and was reduced after training (29%, $p<0.01$)
- Muscle fat reduced 57% in dysglycemics; 27% in controls
- Change of VO$_2$max correlated strongly with change of GIR
**Major findings after 12 weeks training**

- Increased VO2max ~15%
- Increased GIR ~30%
- Dysglycemics reduced body weight (-1.7 kg; \( p < 0.05 \)) and waist circumference (-3.7 cm; \( p < 0.01 \))
- Visceral fat preferentially lost compared to other depots
- Hepatic fat was 5-fold higher in dysglycemics than controls, and was reduced after training (29%; \( p < 0.01 \))
- Muscle fat reduced 57% in dysglycemics; 27% in controls
- Change of VO2max correlated strongly with change of GIR

---

**Table: Changes in Anthropometry and MRI Measurements**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>( \Delta )</th>
<th>Dysglycemia</th>
<th>( \Delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 (17)</td>
<td></td>
<td>53 (10)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>185.3 (9.3)</td>
<td>0.7 (2.5)</td>
<td>178.6 (5.2)*</td>
<td>-1.1 (1.9)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.5 (16.5)</td>
<td>0.2 (0.5)</td>
<td>94.1 (14.1)*</td>
<td>-0.4 (1.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 (3.1)</td>
<td>-0.3 (1.0)</td>
<td>27.8 (5.3)*</td>
<td>-3.5 (5.3)*</td>
</tr>
<tr>
<td>Waist circumference (cm)*</td>
<td>88 (9)</td>
<td></td>
<td>104 (16)*</td>
<td>-3.5 (5.3)*</td>
</tr>
<tr>
<td><strong>MRI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thigh muscle area (mm²/kg)</td>
<td>244 (62)</td>
<td>25 (11)*</td>
<td>264 (55)</td>
<td>26 (20)*</td>
</tr>
<tr>
<td>Adipose depots (mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supraventricular</td>
<td>78 (37)</td>
<td>-3 (11)</td>
<td>118 (60)*</td>
<td>-3 (19)</td>
</tr>
<tr>
<td>Axillary</td>
<td>166 (60)</td>
<td>2 (52)</td>
<td>276 (212)*</td>
<td>-3 (46)</td>
</tr>
<tr>
<td>Pericardial</td>
<td>113 (86)</td>
<td>1 (23)</td>
<td>166 (79)</td>
<td>-2 (30)</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>4776 (896)</td>
<td>-340 (548)</td>
<td>8487 (2878)*</td>
<td>-439 (554)*</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>694 (807)</td>
<td>-215 (396)*</td>
<td>2236 (984)*</td>
<td>-332 (446)*</td>
</tr>
<tr>
<td>Retropertionel</td>
<td>851 (621)</td>
<td>-104 (200)*</td>
<td>1991 (921)*</td>
<td>-131 (203)*</td>
</tr>
<tr>
<td>Inguinal</td>
<td>66 (21)</td>
<td>-2 (16)</td>
<td>106 (59)*</td>
<td>-6 (21)</td>
</tr>
<tr>
<td>Epidermal</td>
<td>6 (3)</td>
<td>1 (2)</td>
<td>9 (3)*</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Popliteal</td>
<td>132 (56)</td>
<td>0 (22)</td>
<td>174 (124)</td>
<td>2 (23)</td>
</tr>
<tr>
<td><strong>Aerobic capacity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO2max (mL·kg⁻¹·min⁻¹)</td>
<td>43.4 (6.9)</td>
<td>6.3 (4)*</td>
<td>38.7 (8.1)*</td>
<td>4.7 (4)*</td>
</tr>
<tr>
<td><strong>Maximum strength</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leg press 1-RM (kg)</td>
<td>188 (58)</td>
<td>25 (20)*</td>
<td>250 (33)*</td>
<td>23 (30)*</td>
</tr>
<tr>
<td>Cable pulldown 1-RM (kg)</td>
<td>70 (18)</td>
<td>10 (9)</td>
<td>75 (18)</td>
<td>10 (10)*</td>
</tr>
<tr>
<td>Chest press 1-RM (kg)</td>
<td>60 (20)</td>
<td>11 (9)*</td>
<td>63 (23)</td>
<td>8 (8)*</td>
</tr>
<tr>
<td><strong>Blood parameters</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F-B-HbA1c (%)</td>
<td>5.2 (0.8)</td>
<td></td>
<td>5.6 (0.6)*</td>
<td></td>
</tr>
<tr>
<td>F-P-Glucose (mmol/L)</td>
<td>5.3 (1)</td>
<td>0.2 (0.4)</td>
<td>5.8 (0.7)*</td>
<td>0.0 (0.6)</td>
</tr>
<tr>
<td>F-S-Insulin (pmol/L)</td>
<td>37.7 (23)</td>
<td>2 (28)</td>
<td>64 (45)*</td>
<td>-0.4 (25)</td>
</tr>
<tr>
<td>F-S-C peptide (pmol/L)</td>
<td>568 (129)</td>
<td>63 (273)</td>
<td>944 (351)*</td>
<td>-46 (425)</td>
</tr>
<tr>
<td>S-CRP (mg/L)</td>
<td>0.8 (1.5)</td>
<td>0.1 (0.7)</td>
<td>1.6 (2.4)*</td>
<td>0.0 (2.2)</td>
</tr>
</tbody>
</table>

*Different from control group \( p < 0.05 \).

^Pre vs. post within group difference \( p < 0.05 \).

*From 17 subjects (control \( n = 8 \), dysglycemic \( n = 9 \)).

bScreening values (post and \( \Delta \) are unavailable).

bNot screening values.
A PGC1-a-dependent myokine that drives brown-fat-like development of white fat and thermogenesis


Francesc Villarroya Irisin, Turning Up the Heat Cell Metabolism 2012, 15, 277 - 8
Evidence against a beneficial effect of irisin in humans

• Raschke et al. *PlosOne*, 2013, 8(9):e73680
  – Mutation in the start codon ATA in stead of ATG, very little transcription of irisin

• Norheim F et al. *FEBS J*. 2014, 281, 739-4
  – No brownin g of WAT with long-term training

  – All 4 antibodies used in ~ 100 papers are unspecific
Interaction between plasma fetuin-A (hepatokine) and free fatty acids predicts changes in insulin sensitivity in response to long-term exercise
Lee et al. Physiol Rep. 2017, Mar;5(5)

- Exercise 12 w reduced plasma fetuin-A conc. (~11%, P < 0.01), slightly changed FFAs concentration, and improved glucose infusion rate (GIR) (~30%, P < 0.01)
- Changes in plasma fetuin-A & FFAs interacted to predict some of the change in GIR (b = 42.16, P = 0.030), AT insulin resistance (b = 0.579, P = 0.003), gene expression of TLR-signaling in AT & AT macrophage mRNA (b = 94.10, P = 0.034) after exercise
- The relation between FFA levels and insulin sensitivity was specific for fetuin-A in AT
- Some effect of exercise on insulin sensitivity may be due to changes in plasma hepatokine fetuin-A and FFAs, → less TLR4 signaling in AT perhaps by modulating AT macrophages
Myokines & adipokines → health & disease

Collaborators

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- Marit Hjort
- Torgeir Holen
- Sindre Lee
- Shirin Pourteymour
- Kristin Eckardt
- Yuchuan Li
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- Bernd Thiede
- Per K. Hol et al.
- Britt Nakstad, Arild Rønnestad, Ola D. Saugstad,
- Jens P. Berg
- Thomas E. Gundersen, Vitas
- Willem de Vos
- NuGO - B van Ommen, H Daniel, M Müller, Robert Caesar
- Food4Me - The Gibneys et al
- NutriTech - B van Ommen et al
- MyoGlu - Birkeland et al.
- Lifebrain - Kristine Walhovd/Anders Fjell
Myokines & adipokines – sum up

• Several 100
• Many are important
  – Irisin is not
• Often expressed in many tissues
• Cooperation between many tissues
• *The truth is rarely pure & never simple*
  
  Oscar Wilde

• Thanks for your attention!